Effect of enzymatic treatments on dietary fruit fibre properties

ANNA CANELA-XANDRI[a], MERCÈ BALCELLS[a,*], GEMMA VILLORBINA[a], MIGUEL ÁNGEL CUBERO[b], RAMON CANELA-GARAYOA[a]

[a]*Chemistry Department-Agrotecnio, University of Lleida, 25198 Lleida, Spain*

[b]*Indulleida, S.A., N. 230, km 12, 25125 Alguaire, Lleida, Spain*

*Corresponding author at: Chemistry Department, ETSEA, University of Lleida, 25198 Lleida, Spain. Tel.: +34 973 702841;

email: balcells@quimica.udl.cat

Keywords: *Bioeconomy, fruit juice industry, waste valorisation, hydrolases, pectinases, SWC, WHC.*

Abstract

Here we studied the modification of fibres derived from the fruit juice industry to prepare new value-added ingredients. These by-products are usually composted or used as animal feed. In this regard, fibres from apple, peach, and citrus fruit were enzymatically treated to improve their functional properties, with the aim to use them as food ingredients. Several enzymes were selected to modify various parts of the polymers present in the fibres. The effect of the treatments was assessed by comparing the water-holding capacity (WHC) and swelling water capacity (SWC) of the modified and raw fibres. FTIR spectrometry was also used to monitor changes in fibre structure. After the enzymatic modifications, some of these fibres not only showed an increase in WHC and SWC compared with the starting material but also greater viscosity.

Introduction

The juice industry generates huge amounts of by-products derived from the pulp, skin and seeds of fruit (Ku and Mun 2008a; Ku and Mun, 2008b; Ling and Hassan 2013, Piarpuzan et al. 2011). Characterised by a high content of many valuable substances (Fernández-Gines 2003; Ku et al. 2008a; Lario, et al. 2004; Sánchez-Zapata et al. 2011), these by-products comprise mainly non-digestible carbohydrates (McKee and Latner 2000; Lario et al. 2004), basically cellulose, hemicellulose, pectin, and lignin (Klemm et al. 2005; Ling et al. 2013; McKee et al. 2000; Piarpuzan et al. 2011; Vergara-Valencia et al. 2007). They may present great structural variability depending on the source and, therefore, the type of fruit (Dhingra et al. 2012; McKee et al. 2000).

Recent years have witnessed considerably increased interest in new applications for food fibres (Peschel et al. 2006). This interest has come about with the implementation of new

technologies capable of either recovering added-value ingredients from by-products or converting them into commercial products, such as raw materials for secondary processes (intermediate food ingredients), operating supplies, and ingredients for new products (Laufenberg et al. 2003; Schieber et al. 2001).

It is well known that the addition of fibre to food can modify its properties, making possible to change the consistency, texture, rheological behaviour and sensory characteristics of the final products where it is used as a functional ingredient (Dreher and Sungsoo-Choo 2001). The addition of fibres to food can reduce production costs, improve quality, flavour and functional properties, as well as shelf life (Fernandez-Gines et al. 2003; Fernández-López et al. 2008; Sánchez-Zapata et al. 2011; Vergara-Valencia et al. 2007; Viuda-Martos et al. 2010). Therefore, fibres are ingredients of great interest to the food industry (Dhingra et al. 2012; Dreher et al. 2001).

However, raw fibres do not always match the desired technological properties needed for food applications. Consequently, these fibres cannot be added to foodstuffs in relevant amounts. In addition, they frequently tend to cause unpleasant textures and colours, thus making their use a challenge (Dreher et al. 2001).

Several modifications of dietary fibre have been studied with the aim to improve functional properties. These properties can be enhanced using chemical (Grethlein, 1991; Gould and Dexter, 1986; Ramaswamy 1991), enzymatic (Caprez et al. 1987; Matsuo 1989) and physical (Giesfeldt et al. 1991) treatments.

In this regard, enzymes have the advantage that they are not toxic, they are effective under mild reaction conditions, they are easy to inactivate, and they can have high effectiveness at relatively low concentrations, In addition, their activity can be regulated by controlling the temperature and pH of the media. However, few studies have addressed the effects of enzymatic treatments on the physicochemical properties of fruit fibre and the results

of these are sometimes contradictory, as different materials, measurement methodologies and process conditions were used (Larrauri 1999).

Here we sought to test the effectiveness of several enzymatic treatments in modifying fruit fibres in such a way as to improve their functional properties. These new materials are expected to increase the applicability of fruit fibres derived from the juice industry, making them a new source of food ingredients. We assessed the effect of the enzymatic treatments by determining water-holding capacity (WHC) and swelling water capacity (SWC).

Material and methods

We used fruit fibres from citrus (CITRUS N, CITRUS M), apple (APPLE N, APPLE M), and peach (PEACH N) obtained from a local fruit juice industry.

Six food grade enzymes were selected, all of them provided by the fruit company. Two were pectin methylesterases (P872L from Biocatalysts and NovoShape from Novozyme)—one from *Aspergillus niger* and the other from *A. aculeatus*, both with pectin methylesterase activity and negligible side activities. Also, two pectinases (P62L and P444L both from Biocatalysts) from *Aspergillus sp*. were used, both with endogalacturonase activity, but with different polygalacturonase:pectin lyase ratios (higher in P62L from Biocatalysts). Finally, a cellulase (CEL13 from Biocatalysts) from *Trichoderma sp* with side activities such as cellobiase, beta-glucosidase and beta-glucanase was used.

ATR-FTIR spectra were recorded using a Jasco FT/IR-6300 spectrometer at a resolution of 16 cm^{-1} and 60 scans in the spectral range of 4000 - 600 cm^{-1}. Water activity (a_w) was recorded at 25°C using an AQUA LAB with an accuracy of ±0.0003 a_w. The pH was measured with a pHmeter (Model 210, Crison 2002, Crison Instruments, S.A.).

Enzymatic treatments

Enzymatic solutions were prepared to meet the working concentration recommended by each manufacturer.

Enzymatic reactions were carried out by preparing a suspension with 15 g of dry fibre in 150 mL of the previously prepared enzymatic solution. The suspension was stirred to achieve a consistent mixture, and the pH was measured. Time and temperature were selected following manufacturer's recommendation for each enzyme. The suspension was kept under agitation at 140 rpm in an automatic shaker for the time indicated for each case.

After the enzymatic treatment, the enzymes were inactivated by heat for the time and at the temperature recommended by the manufacturer. After enzyme inactivation, the pH was measured again.

Treated fibre suspensions were frozen at -80°C for 24 h and lyophilized for 72 h until complete removal of water. Modified fibres were crushed and sieved to obtain a homogenous particle size, and water activity was measured for each sample.

Blank samples were prepared following the same protocol but without the addition of the enzymes.

Characterisation of fruit fibres

Technological properties. Water-holding capacity (WHC) and swelling water capacity (SWC) were determined following published methods (Lario et al. 2004; Robertson et al. 2000). WHC was expressed as g of water held per g of sample, while SWC was expressed as mL/g of sample. Each assay was carried out in triplicate.

In order to compare the effect of enzymatic treatments, % of WHC and % of SWC modification was calculated as stated in Equation 1 and Equation 2:

$$\%\mathbf{WHC_{modification}} = \frac{\mathrm{WHC_{enzymatically\ treated}} - \mathrm{WHC_{non-enzymatically\ treated}}}{\mathrm{WHC_{non-enzymatically\ treated}}} \mathrm{x}\ 100$$

Equation 1: Equation used to calculate % of WHC modification.

$$\%\mathbf{SWC_{modification}} = \frac{\mathrm{SWC_{enzymatically\ treated}} - \mathrm{SWC_{non-enzymatically\ treated}}}{\mathrm{SWC_{non-enzymatically\ treated}}} \mathrm{x}100$$

Equation 2: Equation used to calculate % of SWC modification.

FTIR spectra. FTIR spectra were recorded for each sample once completely dried. Spectra manager software was used to calculate the peak area between adjacent valleys.

Results and discussion

The study was conducted using 5 fibres. APPLE N, PEACH N and CITRUS N were obtained after the juice or puree extraction without any further treatment and are referred to herein as *native* fibres. APPLE M and CITRUS M were obtained from the manufacturer after further processing of the juice waste, respectively, and are referred to as *further processed* fibres.

Effect of enzymatic treatments on the technological properties of fibres

Treatments with pectin methylesterases (PMEs). Treatments with PMEs were performed in deionized water and in tap water. P872L (Table 1) led to considerable changes in the WHC of CITRUS N and APPLE N fibres. CITRUS N fibres showed a greater modification than CITRUS M fibres at each temperature and solvent tested and for most of the reaction times. Similar results were obtained with apple fibres when treated at the lower temperature. In contrast, PEACH N fibre treated with P872L showed little or a loss of WHC improvement.

This result can be explained by the fact that peach fibre has low pectin content, and P872L acts on the ester groups of pectin. Most treatments gave higher modifications at short reaction periods. This behaviour could be a consequence of structural changes in pectins as a result of an increased number of carboxyl groups, which may interact with each other, especially at low pH, thereby providing lower interaction with water and thus less improvement of WHC. The effect of deionized or tap water did not show a clear pattern. The observed differences could be attributable to diverse factors, such as the degree of demethylation, which in turn depends on the pectin content of the fibre and the effectiveness of the PME treatment and, on the other hand, the calcium content of the fruit and also of water in the case of tap water.

The results achieved with NovoShape differed greatly from those with P872L. In general, 60-h treatments gave a greater improvement of WHC than 24-h ones, thereby indicating that the reaction rate of NovoShape was lower than that of P872L. Also, in contrast to P872L, NovoShape treatment of further processed fibres (CITRUS M and APPLE M) led to a greater improvement in WHC than in their native counterparts (CITRUS N and APPLE N). In the case of CITRUS N, an almost negligible improvement was achieved. Peach fibre showed similar results with both P872L and B. Deionized water gave a greater improvement of WHC when the reaction time was 24 h; however, tap water gave the best results in the long term. Similarly to what happened with P872L, the viscosity of the solution increased notably after treatment with NovoShape.

The SWC values after the treatments with P872L (Table 1) were higher with tap water than with deionized water. This result could be explained by the presence of Ca^{2+} ions in tap water, which would promote fibre swelling. However, other than this, we did not find any clear pattern to explain the results in the two types of water. Citric and apple fibres gave better SWC results than peach fibre. NovoShape led to a notable modification in %SWC for all the fibres, except CITRUS N.

It should be noted that treatments with PMEs led to an increase in the viscosity of the solution, which may indicate a certain degree of gelation caused by the interaction between the carboxylate groups released and the Ca^{2+} ions present in the media.

Treatments with pectinases (PECs). APPLE N and PEACH N showed the greatest %WHC modification, but with different behaviours, when treated with P62L (Table 1). While APPLE N results improved with time, those for PEACH N did not. On the other hand, the increase in %WHC in CITRUS N was very low. While a slight depolymerisation may facilitate the interaction with water, excessive depolymerisation could lead to the loss of WHC. Thus, longer reaction times could provide excessive depolymerisation, which would prevent maintenance of an improved WHC.

Higher %WHC values were achieved for further processed fibres than for native ones but the effect of temperature differed in each case. APPLE M presented a small increase in %WHC with increased temperature, while CITRUS M showed a small decrease when temperature increased.

The results achieved with P444L also revealed that the lowest temperature tested gave a greater improvement in WHC especially at longer reaction times, except for CITRUS N.

Modification of %SWC showed a similar pattern to that of %WHC. A shorter reaction time and lower temperature showed, in general, the greatest %SWC modification for treatments with P62L, and the results for further processed fibres were much better than those for native ones. Treatments with P444L did not show a clear pattern. P444L, with a low polygalacturonase (PG): pectin lyase (PL) ratio and also some arabanase activity, led to an increase in %SWC with time in both APPLE N and PEACH N fibres.

Contrary to what happened with PMEs, the viscosity of the solution did not increase in response to PEC treatment, which is in accordance with the fact that PECs action focuses on

the polygalacturonase chains by reducing their length and so rendering fragments that develop less viscosity in solution than in the case of PMEs, in which these chains are not shortened.

Of note, in this case, peach fibre showed a considerable modification of both % WHC and SWC. Although peach fibre has lower pectin content than apple or citrus fruits, two aspects should be taken into account. On the one hand, the pectin content of peach fibre would be easily modified by the enzyme, thereby leading to considerable changes in the structure of the fibre and thus altering its properties. On the other hand, the enzymes used showed arabanase side activity. Consequently, molecules other than pectins can be modified by the action of these enzymes. These modifications could lead to substantial changes in the structure of the fibre and, consequently, in its properties.

Treatment with Cellulase (CEL13). CEL13 generally led to an improvement in %WHC (Table 1). This improvement was lower at higher temperature, except for PEACH fibre. Longer reaction times had the same effect, except for CITRUS N, whose %WHC increased with time. APPLE N showed the best results, with an increase of nearly 40% in WHC when time and temperature were the lowest values assayed. According to the literature, apple fibre is richer in cellulose than citrus and peach fibres (Chen et al. 1988; De Escalada Pla et al. 2012; Lundberg et al. 2014) so a greater effect of cellulases could be expected in this fibre.

When treated with CEL13, APPLE N and PEACH N fibres showed an improvement in %SWC (Table 1), which tended to increase with the temperature and time. However, in the case of CITRUS N, treatment with CEL13 impaired SWC, especially at the highest temperature tested.

Monitoring of enzymatic treatments by FT-IR.

Figure 1 shows the characteristic bands for the FTIR spectra of the fruit fibres before enzymatic treatment. Some characteristic bands were selected and the relationship between their areas was determined for native and selected further processed fibres. Selected bands included:

(1) Band at 3,600 - 3,200 cm^{-1} (O-H stretching vibration)

(2) Band at 2,923 cm^{-1} (Csp^3-H stretching, characteristic of polysaccharides -Yang et al. 2008-)

(3) Band at 1,730 cm^{-1} (methyl ester and acetyl ester groups of hemicellulose -Sun et al. 1998-)

(4) Band at 1,623 cm^{-1} (carboxylate functional group -Sun et al. 1998-)

(5) Band at 1010 cm^{-1} (stretching vibration of C-OH of alcohol groups and carboxylic acids –Seslija et al. 2016-).

The results are summarised in Table 2, which shows the calculated relationship between the areas of FTIR bands. Entries indicated as RAW correspond to raw fibres before enzymatic treatment while the other entries refer to fibres after each enzymatic modification.

Treatments with PMEs. A decrease in the pectin ester band (3) relative to the carboxylic acid band (4) or the Csp3-H band (2) after treatments with P872L or NovoShape (Table 2) would confirm the demethylation of pectin by these enzymes. That is, the greater decrease in the values of either relationship (3)/(2) or (3)/(4) would indicate a greater effect of the enzyme. This decrease can be observed in most of the results in Table 2 for PMEs treatments. On the other hand, the relationship (4)/(2) would be expected to increase as the number of carboxylate groups increases due to the action of PMEs, as was the case.

Treatments with PECs. If we look the (1)/(2) relationship, (OH band (1) relative to the Csp3-H band (2)) in fibres treated with PECs, we can observe a greater value than in the case of raw fibres (Table 2), thus indicating the expected increase in the number of hydroxyl groups when hydrolysing glycosidic bonds. This hydrolysis must release two OH groups for each split bond. The values for the (1)/(2) relationship were generally higher for native fibres (CITRUS N and APPLE N) than for the further processed ones. This observation may indicate that the latter have a lower degree of polymerisation due to further processing and, consequently, the activity of PECs is lower as there is less pectin available for hydrolysation.

Higher values in the relationship (5)/(2) would indicate a greater release of OH groups. For both CITRUS fibres and also APPLE N, P444L, with a low PG:PL ratio and arabanase activity, yielded higher values than P62L, which has a greater PG:PL ratio and no side activity. This finding may indicate that the pectins in these fibres are highly methoxylated and thus PL easily hydrolyses the substrate. However, we cannot rule out that P444L has arabanase side activity and the higher values for these fibres could be linked to the hydrolysis of araban-rich side chains in pectin.

Treatments with CEL13. A clear increase in the relationship (1)/(2) after treatment with CEL13 would indicate an increase in the OH groups present in the media, as expected after the hydrolytic activity of this enzyme on cellulose. Our results were consistent with the hydrolytic activity expected for this enzyme (Table 2).

Conclusions

Treatment of fruit fibre with enzymes with distinct activities led to structural modifications that altered functional properties such as WHC and SWC. Most of the enzymatic treatments

gave rise to an increase in WHC and SWC in most of the fibres. Treatments with PMEs also increased the viscosity of fibre dispersions. Nevertheless, the results were highly dependent on the kind of fibre and the temperature and reaction time used in the treatment. Analysis of selected FT-IR spectra bands helped to confirm some of the expected structural changes when considering the structure of the fibre and the activity of each applied enzyme. The obtained results indicate that enzymatic treatments have a real potential to convert by-products from the fruit juice industry into added-value products.

Declaration of interest statment

The authors report no conflicts of interest. The authors are responsible for the content and writing of this article.

We acknowledge funding from Spanish Goverment (MINECO grant number: RTC-2015-3652-5) and Catalan Government, 2014 SGR 1296 Agricultural Biotechnology research group.

References

Bello-Perez LA. 2007. Fibre concentrate from mango fruit: Characterization, associated antioxidant capacity and application as a bakery product ingredient. Lebenson Wiss Technol 40:722-729.

Caprez A, Arrigoni E, Neukom H, Amado R. 1987. Improvement of the Sensory Properties of Two Different Fibre Sources Through Enzymatic Modification. Lebenson Wiss Technol 20:245-250.

Chen H, Rubenthaler G. L, Leung HK, Baranowski JD. 1988. Chemical, Physical, and Baking Properties of Apple Fiber Compared with Wheat and Oat Bran. Cereal Chem 65:244-247.

de Escalada Pla MF, Gonzalez P, Sette P, Portillo F, Rojas AM, Gerschenson LN. 2012. Effect of processing on physico-chemical characteristics of dietary fibre concentrates obtained from peach (Prunus persica L.) peel and pulp. Food Res Int 49**:**184-192.

Dhingra D, Michael M, Rajput H, Patil RT. 2012. Dietary fibre in foods: a review. J Food Sci Technol 49:255-266.

Dreher ML, Sungsoo Cho S. 2001. Handbook of Dietary Fiber, Marcel Dekker, INC.

Fernandez-Gines JM, Fernandez-Lopez J, Sayas-Barbera E, Sendra E, Perez-Alvarez JA. 2003. Effect of storage conditions on quality characteristics of Bologna sausages made with citrus fiber. J Food Sci 68:710-715.

Fernández-López J, Sendra E, Perez-Alvarez JA, Viuda-Martos M. 2008. Physico-chemical and microbiological profiles of "salchichón" (Spanish dry-fermented sausage) enriched with orange fiber. Meat Sci 80:410-417.

Figuerola F, Hurtado ML, Estevez AM, Chiffelle I, Asenjo F. 2005. Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chem 91:395-401.

Giesfeldt JET, Repta RJ, Deaton IF. 1991. Improved Process for Producing a Dietary Corn Fibre, EP 455259.

Gould JM, Dexter LB. 1986. Modified Plant Fiber Additive for Food Formulations. US 814944. .

Grethlein H. 1991. Dietary Fiber and Process for their Production. US 4997665.

Klemm D, Heublein B, Fink H.P, Bohn A. 2005. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. Angew Chem Int Ed Engl 44:3358-3393.

Ku CS, Mun SP. 2008a. Antioxidant activities of ethanol extracts from seeds in fresh Bokbunja (Rubus coreanus Miq.) and wine processing waste. Bioresour Technol 99:4503-4509.

Ku CS, Mun SP. 2008b. Optimization of the extraction of anthocyanin from Bokbunja (Rubus coreanus Miq.) marc produced during traditional wine processing and characterization of the extracts. Bioresour Technol 99:8325-8330.

Lario Y, Sendra E, Garcia-Perez JF, Sayas-Barbera C, Fernandez-Lopez E., Perez-Alvarez JA. 2004. Preparation of high dietary fiber powder from lemon juice by-products. Innovative Food Science Emerging Technology 5:13-117.

Larrauri JA. 1999. New approaches in the preparation of high dietary fibre powders from fruit by-products. Trends Food Sci Technol 10:3-8.

Laufenberg G, Kunz B, Nystroem M. 2003. Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. Bioresour Technol 87:167-198.

Ling TP, Hassan O. 2013. Effects of aqueous ammonia treatment on fiber's surface morphology and enzymatic digestibility of empty fruit bunch fiber (EFBF). AIP Conference Proceedings 1571:877-881.

Lundberg B, Pan X, White A, Chau H, Hotchkiss A. 2014. Rheology and composition of citrus fiber. J Food Eng 125:97-104.

Matsuo M. 1989. Morphological, Compositional and Physicochemical Properties of "Okara" Fermented by Aspergillius oryzae. J Agric Chem Soc Japan 63:1765-1770.

McKee LH, Latner TA. 2000. Underutilized sources of dietary fiber: A review. Plant Foods Hum Nutr 55:285-304.

Peschel W, Sanchez-Rabaneda F, Diekmann W, Plescher A, Gartzia I, Jimenez D.; Lamuela-Raventos R, Buxaderas S, Codina C. 2006. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem 97:137-150.

Piarpuzan D, Quintero JA, Cardona C.A. 2011. Empty fruit bunches from oil palm as a potential raw material for fuel ethanol production. Biomass Bioenergy 35:1130-1137.

Ramaswamy SR. 1991. Fiber and Method of Making, US patent.

Robertson JA, De MFD, Dysseler P, Guillon F, Amado R, Thibault JF. 2000. Hydration properties of dietary fiber and resistant starch: a European collaborative study. Lebenson Wiss Technol 33:72-79.

Sánchez-Zapata E, Fernández-López J, Peñaranda J, Fuentes-Zaragoza M, Sendra E, Sayas E, Pérez-Alvarez JA. 2011. Technological properties of date paste obtained from date by-products and its effect on the quality of a cooked meat product. Food Res Int 44:2401-2407.

Schieber A, Stintzing FC, Carle R. 2001. By-products of plant food processing as a source of functional compounds — recent developments. Trends Food Sci Technol 12:401-413.

Seslija S, Veljovic D, Krusic MK, Stevanovic J, Velickovic S, Popovic, I. 2016. Cross-linking of highly methoxylated pectin with copper: the specific anion influence. Nouv J Chim 40:1618-1625

Sun R, Fang JM, Goodwin A, Lawther JM, Bolton AJ. 1998. Fractionation and characterization of polysaccharides from abaca fiber. Carbohydr Polym 37:351-359.

Vergara-Valencia N, Granados-Perez E, Agama-Acevedo E, Tovar J, Ruales J,

Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez JA. 2010. Effect of added citrus fibre and spice essential oils on quality characteristics and shelf-life of mortadella. Meat Sci 85:568-576.

Yang C, He N, Li X, Ye M, Zhang C, Shao W, Yao C, Wang Z, Li Q. 2008. The isolation and characterization of polysaccharides from longan pulp. Sep Purif Technol 63:226-230.

Table 1: %WHC and %SWC modification relative to raw fibres for each fruit after enzymatic treatments.

		T	time	CITRUS N		CITRUS M		APPLE N		APPLE M		PEACH N	
				%WHC	%SWC	%WHC	%SWC	%WHC	%SWC	%WHC	%SWC	%WHC	%SWC
P872L	D. W.	30°C	0.5h	38.4	8.5	11.8	5.6	15.6	28.9	-4.7	16.0	4.1	3.2
			2h	2.9	23.7	5.6	21.0	14.0	-9.9	3.8	23.1	-19.9	4.1
		50°C	0.5h	30.5	48.8	15.9	19.5	9.0	-8.5	11.0	-16.1	-0.8	9.7
			2h	27.5	16.4	6.9	-3.5	8.5	-2.2	22.4	43.2	-7.4	-7.8
	T. W.	30°C	0.5h	19.6	24.2	8.2	42.8	4.6	5.0	0.8	29.5	-5.6	-10.9
			2h	4.2	33.5	8.1	9.4	28.3	-20.5	-14.4	1.8	0.5	-15.3
		50°C	0.5h	12.9	37.0	6.9	0.8	10.4	-15.1	37.0	6.4	4.6	-18.8
			2h	23.8	23.4	13.1	3.5	12.3	11.3	28.0	31.5	4.7	-8.8
NovoShape	D. W.	50°C	24h	1.0	-11.0	11.1	38.3	4.0	29.2	30.2	21.5	3.7	21.9
		50°C	60h	0.8	0.2	16.4	30.5	-23.70	73.9	31.4	43.6	6.4	41.3
	T. W.	50°C	24h	1.2	-11.0	13.0	69.3	-6.40	4.1	21.1	8.7	12.3	21.8
		50°C	60h	-	-	23.4	56.5	-	-	53.2	-3.1	-	-
P62L	D. W.	30°C	0.5h	-	-	40.7	114.9	-	-	63.4	93.3	-	-
		50°C	0.5h	2.5	9.6	33.9	77.8	32.9	207.6	75.5	101.4	10.1	25.5
			1h	4.5	-11.4	-	-	36.9	40.8	-	-	0.8	6.6
		55°C	0.5h	-2.0	-32.5	-	-	38.1	-6.6	-	-	3.5	-9.9
			1h	13.9	3.5	-	-	-4.4	14.4	-	-	-7.1	11.3
P444L	D. W.	50°C	4h	17.0	23.3	4.2	-8.0	14.3	38.1	41.5	30.5	9.31	37.6
			8h	-0.9	27.4	-	-	17.6	44.3	-	-	8.1	30.6
			16h	-4.9	10.5	-	-	31.0	50.0	-	-	24.4	60.4
		55°C	4h	8.7	8.9	-	-	12.4	27.1	-	-	1.3	23.3
			8h	11.4	6.8	-	-	12.4	33.7	-	-	6.3	42.5
			16h	-3.6	-37.0	-	-	34.2	92.1	-	-	13.9	25.9
CEL13	D. W.	60°C	2h	-11.27	-8.38	-	-	39.79	19.67	-	-	12.84	48.19
			6h	1.0	0.84	22.6	92.12	13.54	30.33	-12.64	-33.2	8.98	39.42
			18h	16.48	11.61	-	-	12.4	30.73	-	-	2.58	42.52
		65°C	2h	-4.96	-4.38	-	-	24.03	22.66	-	-	13.9	35.94
			6h	-2.64	-1.3	-	-	13.11	32.71	-	-	10.32	55.5
			18h	11.12	-39.04	-	-	12.79	45.96	-	-	2.75	51.3

D.W.: Distilled water; T.W.; Tap water; P872L and NovoShape pectin methylesterase. P62L and P444L: pectinases. CEL13: cellulase.

Table 2: Relationship between the areas of some selected bands in the FT-IR spectra of raw and selected further processed fibres**.**

SOURCE	FT-IR BANDS	(1)/(2)	(3)/(2)	(3)/(4)	(4)/(2)	(5)/(2)
CITRUS N	RAW	28.9	2.0	0.4	5.3	31.7
	P872L		1.8	0.3	5.0	
	NovoShape		1.5	0.4	4.3	
	P62L	33.2				28.0
	P444L	44.5				38.0
	CEL13	49.6				
CITRUS M	RAW	27.0	2.1	0.5	4.6	28.9
	P872L		2.1	0.4	5.4	
	NovoShape		1.7	0.4	4.5	
	P62L	27.8				29.7
	P444L	38.5				35.6
	CEL13	38.6				
APPLE N	RAW	23.0	1.1	0.6	1.8	23.0
	P872L		1.0	0.6	1.8	
	NovoShape		0.9	0.9	2.5	
	P62L	26.2				26.2
	P444L	32.5				32.5
	CEL13	43.3				
APPLE M	RAW	15.0	1.1	0.9	1.2	15.0
	P872L		1.1	0.6	1.7	
	NovoShape		1.3	0.5	2.6	
	P62L	23.1				23.1
	P444L	11.9				11.9
	CEL13	25.8				
PEACH N	RAW	31.1	1.9	0.5	4.1	30.0
	P872L		1.7	0.5	3.6	
	NovoShape		2.1	0.5	4.6	
	P62L	42.5				39.1
	P444L	27.1				38.5
	CEL13	34.0				

The FTIR spectra recorded correspond to: **CITRUS N**: P872L 30°C 0.5 h, NovoShape 50° 24 h, P62L 55°C 1 h, P444L 50°C 4 h; CEL13 60°C 18 h; **CITRUS M**: P872L 50°C 1/2 h, NovoShape 50°C 60 h, P62L 30°C 0.5 h, P444L 50°C 4h; CEL13 60°C 6 h; **APPLE N**: P872L 30°C 2 h, NovoShape50°C 60 h, P62L 55°C 0.5 h, P444L 55°C 16 h, CEL13 60°C 2 h; **APPLE M:** P872L 50°C 2 h, NovoShape 50°C 60 h, P62L 50°C 0.5 h, P444L 50°C 4 h, CEL13 60°C 6 h; **PEACH N:** P872L 50°C 0.5 h, NovoShape 50°C 24 h, P62L 50°C 0.5 h, P444L 50°C 16h, CEL13 65°C 2 h. The RAW entry corresponds to the mean of the FTIR spectra recorded for each fibre before enzymatic treatment. P872L and NovoShape: pectin methylesterases. P62L and P444L: pectinases. CEL13: cellulase.

Figure 1: FTIR-ATR for raw fibres. Peaks selected for comparison are indicated.